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VARIETAL SUSCEPTIBILITY OF POTATOES TO INTERNAL BLACK SPOT[1]

P. H. MASSEY, JR., H. C. THOMPSON AND ORA SMITH

Potato tubers may develop many different types of internal blackening. Internal black spot should not be confused with internal brown spot as described by Ellison (2), after-cooking blackening, or discoloration resulting from pathogen invasion. Thompson (10) reports that black spot appears to be a physiological defect involving a breakdown of the tissue just beneath the skin of the tuber. This condition of potatoes was first reported by Horne (3) in 1912, in England. He called it "bruise" since bruising was necessary for the appearance of the black or bluish-black spots. This same problem was worked on in Holland by Oortwijn Botjes and Verhoeven (5) in 1927, and by Oortwijn Botjes (6), de Bruyn (1), Verhoeven (11), and Waal (12), in 1929. These workers called the tuber discoloration "the blue disease of potatoes." Their work indicated that there are varietal differences in susceptibility to blackening and that high rates of potash application would reduce the incidence of black spot.

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The blackening is usually limited to the outer one-fourth inch of cortex just beneath the skin. In most varieties the darkening cannot be detected prior to peeling and the spots tend to be concentrated near the stem end and on prominent points. Wiant (14) emphasized that pressure bruised areas (flaccid spots) were particularly susceptible. Jacob, White-Stevens and Smith (4) found that the tubers become predisposed to blackening prior to harvest and that bruising is the most important factor in the development of black spot. If the predisposed tubers are not bruised, no blackening occurs.

Black spot was first reported in this country in Suffolk County, Long Island, during the 1938-1939 storage season, by Smith (9). It has now been found in Upstate New York, New Jersey, Pennsylvania, Maine and in the Red River Valley of Minnesota and North Dakota. Since 1940, extensive field and storage experiments have been conducted at the Long Island Vegetable Research Farm and in Ithaca, New York, in an effort to solve this problem. Studies have been made on the effect of major and minor fertilizer elements, soil reaction, irrigation, variety, date of planting and storage conditions.

White-Stevens and Smith (13) in 1944-1945 found Green Mountain to be the most susceptible variety of several tested. They concluded that bruising was distinctly modified by some of the above-mentioned factors. Bruising caused more blackening in potatoes stored in bank storage than in cellar or barn storages and more in those grown with a high level of nitrogen or low potash.

Scudder, Jacob and Thompson (7) verified the fact that high rates of potash application reduce blackening and that varieties differ considerably in susceptibility to black spot. They further stated that the varieties gave about the same relative response each year; Green Mountain, Teton and Ontario consistently blackened more than any other variety tested.

Of all the factors studied, variety, late planting and high potash application have given the most promising results. Scudder (8) found variety to be the most important single factor consistently influencing black spot susceptibility. He considers the use of less susceptible varieties to be the most practical method of preventing serious losses on soils with tendencies toward the production of blackened tubers.

Many workers have reported a relationship between the specific gravity of tubers and their susceptibility to blackening. De Bruyn (1) showed that potato tissue with a high specific gravity is more susceptible to bruising injury and that the stem end portion of the tuber has the highest specific gravity. Scudder, Jacob and Thompson (7) observed a high correlation between black spot and specific gravity. They found that high quality (high specific gravity) tubers were most frequently susceptible to blackening, but state that this is not always the case.

The authors thought it advisable to further test the above relationship and to obtain additional information on the blackening susceptibility of some new and standard potato varieties when grown in different locations in this state.

Materials and Methods

1949-1950 Specific Gravity Test

This experiment was designed to further test the relation between

specific gravity and black spot index. Approximately three-hundred pounds of Long Island-grown Katahdin potatoes were used for each of two replications. These were harvested in October and brought to Ithaca and separated into nine specific gravity ranges of 0.005 unit each, using salt solutions calibrated with a hydrometer. The samples were then placed at 40°F. In February the tubers were removed from storage and bruised in a mechanical bruiser designed to afford equal bruising for all lots. The samples were returned to the storage room immediately following the bruising. Two days later the potatoes were peeled and observations were made to determine the blackening index of each lot.

The rating system used was originated by Scudder (8). Ratings were made on a 0 to 9 scale (9 being severe blackening). The percentage black $\left(\frac{\text{number of tubers black}}{\text{total number of tubers}}\right)$ was determined and finally the blackening index $\left(\frac{\text{per cent black X rating}}{10}\right)$. The authors consider the blackening index to be the best measure of blackening since it is composed of the per cent of tubers that blacken as well as the intensity rating.

1949-1950 Variety Test

A variety study was made in widely separated areas using variety trial material from a uniform seed source. Ashworth, Green Mountain, Katahdin, Ontario, Pontiac and Teton varieties were used in this test. They were planted in five counties: Essex, Genesee, Onondaga, Suffolk and Tompkins.

Fifty-pound tuber samples were obtained in October from each field replication of the potato variety trials of Dr. E. V. Hardenburg. In each location he used a randomized block design with four replications. A 5-10-10 commercial fertilizer was applied at the rate of 1500 pounds per acre. Each fifty-pound sample was sub-divided into four small samples (a, b, c, d) and the specific gravity was taken on each of these by the weight in water technique and then they were placed in 40°F. storage.

The potatoes were removed from storage and bruised in the mechanical bruiser and returned to their storage two days prior to peeling. The "a" samples were bruised, peeled and rated in January. The statistical analysis of the data included split plot variance analysis of the percentage of blackened tubers, the black spot index and the specific gravity. The counties were then combined and a correlation analysis was run on the specific gravity and the black spot index.

1950-1951 Variety Test

The previous experiment indicated that additional work would be needed to determine further the effect of different locations on black spot and specific gravity. This season the authors tested Ontario, Teton, Katahdin, Kennebec, Sebago, Ashworth, Cobbler, Essex and Green Mountain varieties grown in Franklin, Madison, Oneida, Suffolk, Tompkins and Monroe Counties.

In October, a twenty tuber sample was saved from each of three field replications of the late Dr. E. V. Hardenburg's potato variety trials. The experimental design and fertilizer application were the same as in the

previous experiment. The specific gravity was taken on all samples individually and then they were placed in storage at 40°F.

Two days before peeling, the potatoes were removed from storage, bruised and returned to the storage. In February, all samples were peeled and tuber ratings were made on the blackening.

Results

1949-1950 Specific Gravity Test

A correlation analysis was made on the data to test the relation between black spot index and specific gravity. Figure 1 shows the correlation of specific gravity with black spot index. A highly significant positive correlation of .933 was found between these two variables.

The results indicate that within the Katahdin variety, the tubers with high specific gravity are more susceptible to black spot. This further verifies the results of Scudder, Jacob and Thompson (*7*). As they pointed out, however, this relationship does not always hold between varieties. The Teton variety has a relatively low specific gravity and yet blackens severely. The Kennebec variety is relatively mealy and blackens only

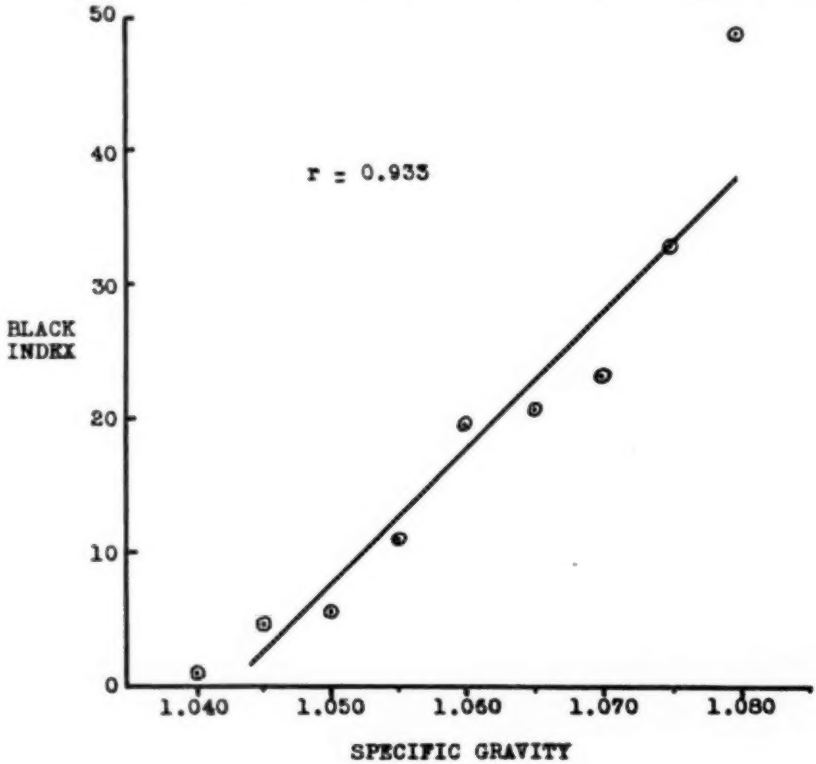

Figure 1. Correlation of specific gravity with black spot index. 1949.

slightly. For this reason there seems to be hope for a potato breeding program to succeed. Dr. L. C. Peterson is conducting such a program at Cornell in an effort to produce a high quality potato that does not blacken.

1949-1950 Variety Test

The results of this experiment show the effect of field location on the relative response of the various varieties with regard to both black spot and specific gravity. Of the potatoes tested in five counties, those from Onondaga showed the most blackening and from Essex the least. Table 1 shows the results from these two counties. Since potatoes from the other counties were intermediate in severity of blackening their results are not shown.

In Onondaga County the Ontario variety blackened significantly more than Pontiac, Ashworth and Green Mountain. A highly significant reduction in discoloration was obtained with the Pontiac and Ashworth varieties under Ontario, Teton, or Katahdin. Ontario blackened significantly more than most other varieties and yet possessed about the lowest specific gravity of any of the tested varieties.

TABLE 1.—*Average blackening index and specific gravity in Onondaga and Essex Counties. 1949.*

Variety	Means of 4 Replications			
	Index		Specific Gravity	
	Onondaga	Essex	Onondaga	Essex
Pontiac	14.3	2.3	1.062	1.059
Ashworth	20.6	0.9	1.069	1.060
Green Mountain	28.2	4.8	1.078	1.067
Katahdin	42.2	10.9	1.073	1.066
Teton	42.3	4.2	1.073	1.063
Ontario	50.1	29.9	1.065	1.060
L.S.D. .05	13.2	5.8	.004	.003
L.S.D. .01	18.3	8.0	.006	.005

In Essex County Ontario gave considerably more blackening than any other variety tested, even though its specific gravity was significantly less than that of Green Mountain and Katahdin. The Ashworth and Pontiac varieties were again found to be the least susceptible to internal discoloration.

The "a" samples that were peeled in January showed more discoloration than the b, c, and d samples that were peeled in March. These results are in agreement with the results of other experiments by the authors which show that blackening gradually increases in storage from November to February and then tends to drop-off slightly.

New York State was very dry and hot during this season and the specific gravity of all the varieties was much below normal expectations. Figure 2 shows the response to blackening of four varieties in the locations tested. It can be observed that the varieties responded similarly in relation to each other at all locations. Ontario was the most susceptible and Pontiac the least susceptible to discoloration in all counties.

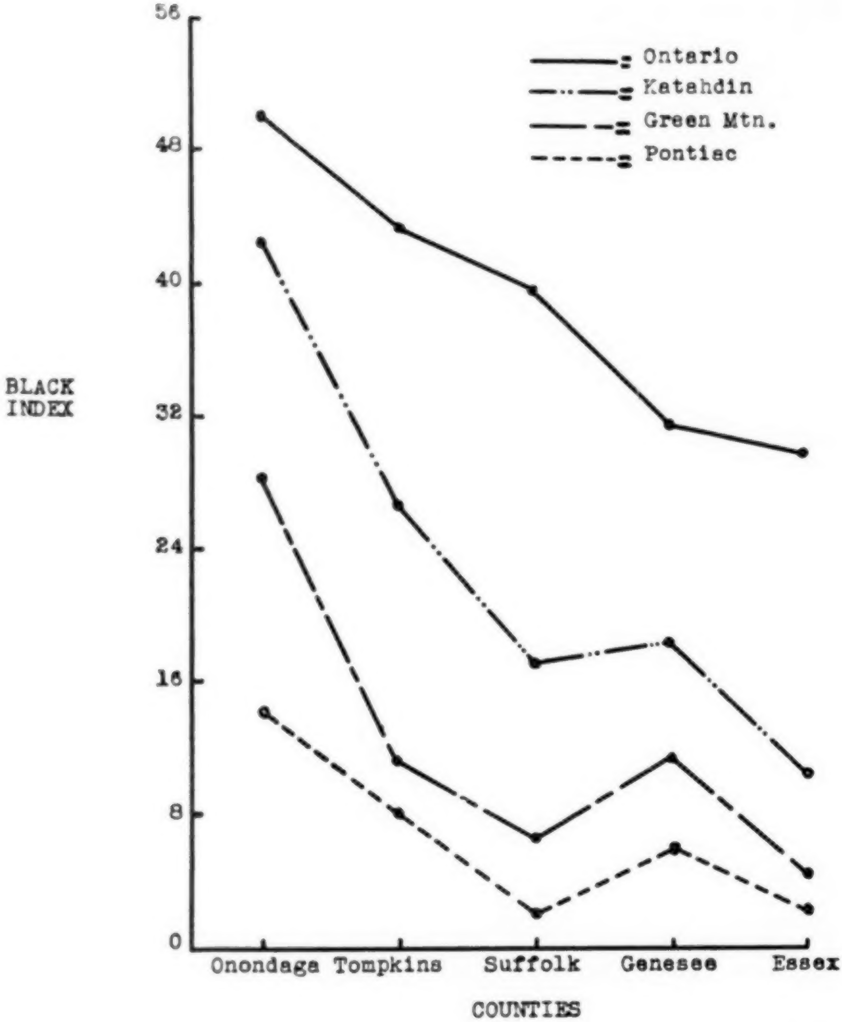

Figure 2. Average blackening indices of Ontario, Katahdin, Green Mountain and Pontiac in five counties in 1949.

In the combined analysis of all counties, a highly significant total correlation of +.286 was found between specific gravity and black spot index. Upon adjusting the mean black spot index values to a common specific gravity, the ranking of the variety means left the order unchanged. However, the adjustment of the county means changed the county order somewhat. Ranking third in severity of discoloration prior to adjustment, Suffolk County after adjustment, was increased in blackening to the degree

found in most seasons. The analysis of covariance also showed varieties and counties to be highly significant after removing the effect of specific gravity indicating that other factors are also influencing blackening.

1950-1951 Variety Test

To show the response of the varieties in the various locations the authors again selected two extreme counties, Suffolk and Franklin. The results of these counties can be seen in table 2.

Suffolk County had a much higher percentage of blackened tubers and also larger and more intense tuber discoloration than the other locations. Ashworth blackened significantly less than Cobbler, Kennebec, Green Mountain, Teton and Ontario. Ontario blackened considerably more than any other variety tested in this county. It should be pointed out that although Kennebec was intermediate in blackening in Suffolk County, in all other locations it showed very little tendency to blacken.

In Franklin County the Teton variety blackened more than Ashworth, Essex, Kennebec, Green Mountain, Katahdin and Sebago to a highly significant degree. All of the varieties showed less discoloration in this county than they did when grown in the other locations.

TABLE 2.—*Average blackening index and specific gravity in Suffolk and Franklin Counties. 1950.*

Variety	Means of 3 Replications			
	Index		Specific Gravity	
	Suffolk	Franklin	Suffolk	Franklin
Ashworth	0.5	0.2	1.066	1.066
Sebago	5.2	3.9	1.072	1.074
Katahdin	8.4	2.8	1.072	1.072
Essex	10.7	0.9	1.069	1.066
Cobbler	15.9	6.4	1.072	1.075
Kennebec	24.4	1.7	1.075	1.080
Green Mountain	32.7	2.7	1.091	1.083
Teton	36.9	16.5	1.076	1.070
Ontario	85.5	9.7	1.079	1.072
L.S.D. .05	9.3	8.5	.006	.004
L.S.D. .01	12.8	11.7	.008	.006

Figure 3 shows the results of the county x variety interaction. Several of the varieties reacted differently in certain locations. For example, Cobbler and Katahdin showed more blackening than Ontario in Tompkins County. In Oneida County, Katahdin showed less discoloration than Ashworth which had the lowest blackening index in all other locations.

In the combined analysis a highly significant total correlation of +.468 was found between specific gravity and black spot index. Adjustment to a common specific gravity of the county black spot index values resulted in very little change in the county blackening rank. Adjustment did cause some shifts in position of the variety means; Kennebec was reduced in blackening rating and Essex was increased. The analysis of covariance showed, as in the 1949 results, that varieties and counties were significant after removing the effect of specific gravity. This is further evidence that other factors are also influencing blackening.

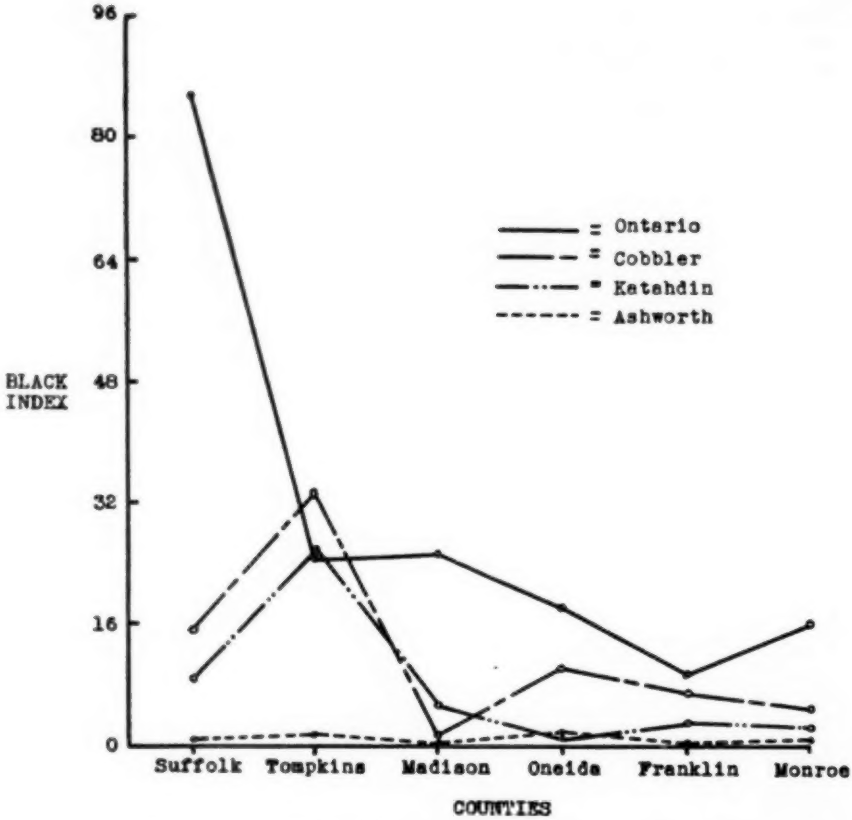

Figure 3. Average blackening indices of Ontario, Katahdin, Cobbler and Ashworth in six counties. 1950.

An analysis of error variance indicated an increase in precision of the experiment by considering the independent variable (specific gravity).

Summary

1. Experiments were conducted to further test the correlation between black spot index and specific gravity, and to show the effect of field location on the relative response of various varieties to black spot and specific gravity.
2. A highly significant positive corelation of .933 was found between black spot index and specific gravity in 1949, using Long Island-grown Katahdin potatoes. We must conclude that there are other factors influencing blackening, but that there is an association between the specific gravity of the tuber and the black spot index.
3. The variety trials in 1949-1950 indicate that internal black spot is no longer just a Long Island problem, but is becoming increasingly prevalent throughout New York State.

4. The fact that location has an important effect on the incidence of black spot was demonstrated in the 1950-1951 trials. Several of the tested varieties reacted differently in blackening susceptibility when grown in certain counties. However, Ontario consistently blackened more than the other varieties in practically every location, whereas Ashworth, Pontiac and Kennebec showed the least susceptibility to discoloration of any of the varieties tested.
5. The most practical recommendations for the control of internal black spot are: (1) careful handling to avoid bruising, especially after very long storage and (2) the use of less susceptible potato varieties.

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THE EFFECT OF TILLAGE PRACTICES ON THE YIELD OF IRISH COBBLER POTATOES[1]

WALTER C. JACOB[2] AND M. B. RUSSELL[3]

Intensive cropping systems have been used by vegetable growers on Long Island for many years. The combination of this intensive cropping and the increasing use of heavy machinery have resulted in the formation of a zone of compaction at a depth varying from eight to twelve inches. This results in impeded drainage and restricted root penetration. Root studies have shown that very few potato roots penetrate the compacted layer and it has been thought that increased drought resistance might be obtained if the roots could penetrate deeper. The underlying soil horizons are highly permeable sand and gravel and many growers have adopted the practice of breaking the "hard pan" mechanically with various tillage implements. These growers report improved drainage and increased yields from these operations. Evaluation of these claims cannot be made because no untreated control areas are available for making comparisons.

Preliminary studies have shown that the bulk density of soil from the zone of compaction may be as high as 1.75 gm. per cc. and that the rate of water penetration decreases rapidly when the bulk density of the soil exceeds 1.5 gm. per cc. This zone of compaction can also be detected by a penetrometer (1). In the data given this zone seemed to be in the region from 6 to 9 inches in depth.

A survey of the growers on Long Island indicated that several different tillage implements were being used to break up this "hard pan." Since no information was available with which to evaluate the various implements it was necessary to include all of them in the same experiment.

In 1947 an experiment was begun with the primary objective of obtaining estimates in the form of crop yield of the influence of the use of the Killifer subsoil chisel, the "jostler" subsoil cultivator, the TNT subsoil plow, the conventional moldboard plow and the disk harrow with and without supplemental irrigation. The experiment was designed to continue for a minimum of four years on the same plots and this paper is a report of the results.

MATERIALS AND METHODS

The experiment was located on a block of land of the Long Island Vegetable Research Farm which had been treated uniformly and which had had no previous deep tillage operations. Checks were made to make certain that the compacted zone existed in this area of land.

Early potatoes were selected as the crop to be grown and certified seed of Irish Cobbler variety was used. All seed was treated for seedpiece decay and was cured after cutting. The seed pieces in this test were planted

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12 inches apart in 34-inch rows and fertilizer was banded in at planting time. In 1947 and 1948, 2000 pounds of 5-10-5 fertilizer containing 50 pounds magnesium per ton were used and in 1949 and in 1950 this rate was increased to 2500 pounds per acre. Each fall after harvest a rye cover crop was sown. This was plowed under in the spring. Insects and diseases were controlled with standard commercial methods.

The treatments were combinations of all levels of the following factors:

1. *Irrigation*
    1. Irrigation at the rate of one inch per week minus the rainfall of the preceding week.
    2. No supplemental irrigation.
2. "*Killifering*" (performed in the late fall to depth of 20 inches)
    1. None
    2. Chiseled at 2-foot intervals every 4 years.
    3. Chiseled at 4-foot intervals every 4 years.
    4. Chiseled at 2-foot intervals every year.
    5. Chiseled at 4-foot intervals every year.
3. *Primary tillage* (performed in early spring before planting)
    1. Disk only, 3-4 inches deep.
    2. Plow 8 inches deep.
    3. TNT plow 8 inches plus 4 inches deep.
4. "*Jostling*" (performed during the growing season to depth 14 inches)
    1. No jostling.
    2. Jostled once during growing season (first cultivation).
    3. Jostled twice during growing season (first and third cultivation).

Because of the nature of the treatments it was necessary to use a split plot design. There were two complete replications, each split in half for the two levels of irrigation. At right angles to this split each large irrigation plot was split into 5 plots for the killifer treatments in the fall across the direction of the rows. Each large irrigation plot was divided into three plots for the primary tillage operations, these at right angles to the killifer plots. The primary tillage plots were further divided into three plots for the jostling treatments. Each final plot was 4 rows wide and 52 feet long. To provide border areas, records were taken from the center 30 feet of the two middle rows of each plot. There were 180 plots in the experiment.

All data analyzed by the analysis of variance technique for the split plots and only significant effects will be presented.

Results

During the four years in which the experiment was conducted, disking was characterized by producing significantly fewer potatoes than either of the types of plowing. The average yields for the four years are presented in table 1. A reduction, on the average, of 60 to 70 bushels per acre was the comparison of disking with the two types of plowing. It made little difference whether the ordinary moldboard plow or the TNT plow was used.

TABLE 1.—*The influence of methods of primary tillage operations on the yield of Irish Cobbler potatoes.*
(Ave. of 4 yrs.)

Type of Primary Tillage	Yield of U. S. No. 1 Size Bus. per Acre
Disk only — 3-4 inches deep	261
Plow — 8 inches deep	330
TNT plow — 8 plus 4 inches deep	323
L.S.D. @ P ≤ .05	11

TABLE 2.—*The influence of frequency and intensity of Killifer operation on yield of Irish Cobbler potatoes.*
(Ave. of 4 yrs.)

Killifer Frequency and Intensity	Yield of U. S. No. 1 Size Bus. per Acre
None	320
Every 4 years at 2-foot intervals	309
Every 4 years at 4-foot intervals	300
Every year at 2-foot intervals	276
Every year at 4-foot intervals	317
L.S.D. @ P ≤ .05	18

TABLE 3.—*The influence of "jostling" frequency on the yield of Irish Cobbler potatoes.*
(Ave. of 4 yrs.)

"Jostling" Frequency	Yield of U. S. No. 1 Size Bus. per Acre
None	306
Once — at first cultivation	303
Twice — at first and third cultivation	304
L.S.D. @ P ≤ .05	12

None of the differences between the killifer treatments was significant on a yearly basis. However, on the average for four years the consistency of reduction of yield by the 2-foot intervals every year treatment led to a significant difference as revealed in table 2.

There were no differences in yield caused by the "jostiing" treatments (Table 3) on the average for 4 years. This lack of difference was quite consistent during the years also.

TABLE 4.—*The influence of irrigation on the yield of Irish Cobbler potatoes in different years. (Bus. U. S. No. 1 size per acre)*

Year	Irrigation Level None	Irrigation Level 1 Inch	Increases Due to Irrigation
1947	354	342	—12
1948	314	274	—40
1949	110	243	133
1950	368	423	55
Least significant increase @ P ≤ .05			—70

Except for 1949, irrigation had no marked effect on the yield of potatoes (Table 4). However, irrigation figured quite prominently when considered in combination with certain other factors.

While the main effects of the factors already pointed out were quite consistent, certain effects were changed by the level of other factors. In figure 1 is given, graphically, the influence of primary tillage operation on the effect of irrigation on the response to different frequency of killifering in different years of Irish Cobbler potatoes. In general the use of the killifer treatment every year resulted in poorer yields than did the treatment once in four years. However, with irrigation, the use of the killifer treatment every year was better than every four years, when the TNT plow was used. This response varied some with years but except for 1950 the response

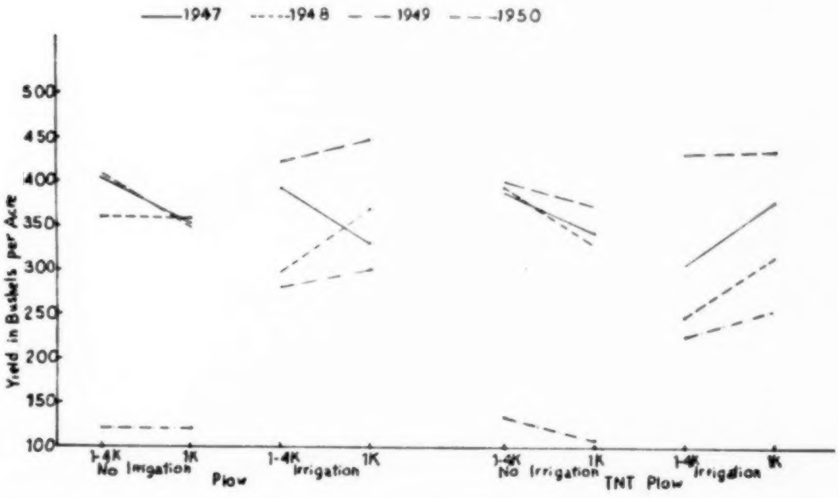

Figure 1. The influence of primary tillage operations on the effect of irrigation on the response of Irish Cobbler potatoes to different frequency of killifer operations in the different years.

was quite marked. When a moldboard plow was used with irrigation, 1948 was the only year when "every-year killifer" was better than every four years. In other years there was no difference, showing that every year was poorer than every four years.

Discussion

The fact that disking alone gave fewer potatoes than did plowing was no surprise to potato growers. The potato does best with loose soil at planting time and the disking was definitely inadequate seed bed preparation. There was some thought that the combination of disking with deep tillage under irrigation might be satisfactory but such was not the case here.

Because the only response to killifer treatments was reduction in yields when the land was chiseled too often too close together there is considerable doubt as to the advantage of breaking up the "hard pan" mechanically for potatoes. The hard pan existed at the beginning of the experiment and no benefits could be derived from breaking up this pan. It may be that mechanical action of this type is of too short duration to do any good. Since killifer is used in the fall the hard pan may well be reformed by summer when the potato roots have reached to that depth. It would seem that growers should at least check themselves in the future to determine whether the deep tillage is of economic value to them.

The change from a decreased yield to an increased yield by the annual killifer method compared with every four years when the TNT plow was used with irrigation might indicate some reason for the generally poorer yields for the more frequent killifer treatment. If the increased drainage resulted in droughty conditions then irrigation might overcome this. It is to be noted, however, that killifering every year with irrigation was no better than killifering every four years without irrigation except in 1949 when the response to irrigation was so great.

The significant differences between killifering every year and every four years which showed up in 1947, the first year of the experiment, when these two treatments were really identical has caused some question. No apparent soil differences were in the plots which would have caused these differences in yield and even though there were only two replications there were many more plots of these treatments because of the factorial nature of the experiment. Certainly more work is needed on the subject of deep tillage for potatoes.

Summary

1. Various types of tillage implements including the killifer sub-soil chisel, the jostler subsoil cultivator, the TNT subsoil plow, the conventional moldboard plow and the disk harrow were evaluated as to their influence on the yield of Irish Cobbler potatoes over a four-year period.
2. Disking only consistently reduced the yield of potatoes compared to plowing, or TNT plowing.
3. The only significant effects of killifer treatments was a reduction in yield of potatoes when the frequency and intensity were too great.
4. Except for 1949 there was no significant increase in yield by irrigation.

5. Annual killifer treatments seemed to be more beneficial when combined with irrigation and TNT plowing than killifering once in four years.
6. More studies regarding the economics of deep tillage operations in the Northeast need to be conducted.

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A MACHINE FOR PLANTING POTATOES IN TUBER UNITS[1]

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In 1950 a machine for planting potatoes in tuber units was constructed by William F. DeBerry, a potato grower at Oakland, Maryland, in Garrett County, in cooperation with the writer. This machine was built so it could be easily substituted for the planting unit in an Iron Age potato planter.

A small plow is inserted in front to open up a furrow and in the rear two discs are arranged to close the furrow after the seed pieces have been planted. The cutting knife is stationary and is made with two blades crossing at right angles. The potatoes are placed on the knife by the operator from a box attached to the planter. They are then cut into four pieces by a weight raised by a chain attached by a clutch mechanism to the axle of the planter wheels. This weight is attached to a block of wood cut so that slits fit over the knife while the tuber is being cut. The force of the dropping weight cuts the tubers into four pieces each of which falls through a separate tube into the furrow which was opened by the plow. The tubes are arranged so the pieces are spaced equidistantly in the furrow. Provision is made to maintain automatically a uniform distance between the units. Two nozzles are attached in order to spray the knife with a disinfecting solution after each tuber is cut. The solution is sprayed by the operator with an atomizer bulb. Fertilizer can be applied with the attachment on the planter.

During the 1950 and 1951 seasons approximately ¼ acre of land was planted with this planter which was pulled slowly but steadily by a tractor. After the potatoes emerged the units could be easily recognized and diseased units could be readily detected and removed.

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THE EFFECT OF 2,4-D TREATMENT ON FREE AMINO ACIDS IN POTATO TUBERS[1]

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A number of workers have studied the effect of natural and synthetic plant hormones on nitrogen metabolism. Recently Sell *et al.* (15) have stated that red kidney bean plants, treated with a phytotoxic level of 2,4-D accumulate nearly twice as much crude protein in their stems as non-treated plants. They found that by the microbiological methods of Stokes *et al.* (24) and Wooley and Sebrell (26), that 11 amino acids accumulated in treated stems. Although not stated by the authors, it is presumed that this accumulation included free amino acids and amino acids from protein hydrolysis. These data were interpreted by the authors to mean, that there had been a change in the character of the proteins of treated plants because of the variation in the proportions of the amino acids. This interpretation is questionable, since the samples presumably included the free amino acids as well as amino acids from protein hydrolysis. It is possible that the changes were due to free amino acids alone; thus the treatment might not have changed the protein pattern. Conversely, the increase in total amino acids in the treated plants might have been due exclusively to the amino acids from the hydrolyzed proteins.

Several workers have studied the effect on nitrogen metabolism of natural and synthetic plant hormones. Smith *et al.* (16) using the Ma and Zuazaga method (7), and Hamner (8), Stuart (25), and Rhodes *et al.* (14), using the A.O.A.C. method, have reported definite increases in total nitrogen, exclusive of nitrates in stems of treated bean plants. Smith *et al.* (17), using the A.O.A.C. method, found definite increases in total nitrogen including nitrates. Borthwick *et al.* (1) found increased protein in roots and tops of treated tomato plants by a method using Millon's reagent. Smith *et al.* (16) using micro-Kjeldahl total nitrogen, found increased total nitrogen exclusive of nitrates in stems and roots of treated bindweed plants *(Convolvulus arvensis)*.

Rasmussen (13) found both increased soluble nitrogen and increased protein nitrogen in the roots of summer-treated dandelions *(Taraxacum officinale)* using the reduced iron method. Since fall treatments resulted in decreased soluble nitrogen and increased protein nitrogen, the time of treatment is apparently a significant factor.

Erickson *et al.* (5) have reported an increased percentage of crude protein in wheat from plants treated with 2,4-D. The method was not stated. This may have been an actual increase in protein per seed or simply a decrease in stored polysaccharides. Decreases in stored polysaccharides due to 2,4-D treatment is a well established fact according to the work of Rasmussen (13), Mitchell and Brown (9).

Recently Stahler and Whitehead (18) have reported excessive accumulation of nitrates in leaves of sugar beets sprayed with 2,4-D, by a method not stated. These facts, taken collectively, suggest that increases

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in total nitrogen, soluble nitrogen and protein nitrogen may be directly or indirectly due to increased nitrate assimilation.

Rakitin and Troyan (12) treated tubers with the methyl ester of alpha naphthalene acetic acid for the purpose of sprout inhibition. This also caused an accumulation of amino nitrogen. Their method is not stated in the English abstract.

The purpose of the present paper is: (1) to report the changes in free amino acid patterns of potato tubers, due to treating the plants with 2,4-D; (2) to suggest a possible mechanism of 2,4-D action which could explain these changes.

METHODS.—The Red McClure potatoes used were selected from the field test samples described by Payne *et al.* (11), and from similar samples produced in 1951. Only the data for the 1950 samples are presented here since results were similar. Treated plants received a single spray application of ⅓ pound per acre of the sodium salt of 2,4-D. Twenty treated and twenty control tubers were first frozen, then allowed to thaw, after which they were washed and dried. The juice from all control tubers was squeezed into a volume of 95 per cent ethyl alcohol until the final alcohol concentration was 80 per cent. The treated tubers were handled similarly. The two solutions were allowed to boil about 1 minute, then after cooling, the proteins were filtered out.[3]

The filtrates were evaporated over a hot water bath with a fan in motion to keep the solutions relatively cool. This procedure was used until the filtrates were concentrated to one-fifth of their original volume. The filtrates contained soluble nitrogen compounds and sugars. No effort was made to remove sugars, since they are not reactive with ninhydrin.

The technique of paper partition chromatography was followed. This procedure was devised originally by Consden *et al.* (2) and later used on potato extracts by Dent. *et al.* (4).

For one dimensional chromatograms, 1 lambda[4] of the concentrated filtrate was placed on Whatman No. 1 filter paper (size 16" x 16"). Four 1-lambda samples of the control concentrate and 4 of the treated were placed on each of 4 sheets. The 4 sheets were run in separate jars. The mean densichron readings shown in table 2 represent 14 determinations for each amino acid spot. The solvent used was reagent grade phenol (Baker and Adamson) saturated with water. A few crystals of potassium cyanide were added to each jar to prevent the oxidation of the phenol and occasionally a drop of ammonium hydroxide to bring out the histidine spot. Chromatography was continued at room temperature until the solvent had nearly reached the top of the paper. The papers were removed and dried over night at room temperature. Amino acid spots were revealed by spraying the dried sheets with 0.1 per cent ninhydrin in 95 per cent alcohol (Stepka and Takahashi, 19). Several drops of glacial acetic acid were added to eliminate alkalinity, which prevents full development of the ninhydrin colors.

Relative densities of the amino acid spots were determined 18 hours after spraying by use of a Welch Densichron No. 2150 with a green N

[3]It was thought, at first, that such heat treatment might cause a loss of some amino acids as cited by the work of Patton (10). A critical study was therefore made (data not included), and it showed that the amount of heat here did not change the results.
[4]Microchemical Specialties Co. No. 282-C.

filter. The color developed by spraying with ninhydrin reaches its maximum about 18 hours after spraying and remains fairly constant for several hours before starting to fade. The amino acids in the spots of the one dimensional chromatograms were identified by running spots of the pure amino acids alongside the potato juice, and by separate two dimensional chromatograms run in a phenol-collidine system. The amino acids of the two dimensional chromatograms were then identified by their Rf values, their pattern on the chromatograms, and a spotting technique suggested by Dent (3) and by Dent *et al.* (4).

TABLE 1.—*Free amino acids found in Red McClure potatoes by 2-dimensional paper partition chromatography.*

A. Free Amino Acids in Potato Juice (Original Volume 5 λ Spots)		B. Free Amino Acids in Potato Juice (Concentrated ⅕ Volume 5 λ Spots)		C. Free Amino Acids in Potatoes Found by Dent, Stepka and Steward (10 mg N/1 ml)	
Aspartic acid	(2)	Aspartic acid	(2)	17 Amino acids found in B.*	
Glutamic acid	(3)	Glutamic acid	(3)		
Serine	(4)	Serine	(4)	Tryptophan	(20)
Asparagine	(6)	Asparagine	(6)	Glycine	(5)
Alanine	(8)	Alanine	(8)	Alpha amino butyric acid	(10)
Glutamine	(9)	Glutamine	(9)	Cysteic acid	(1)
Lysine	(12)	Lysine	(12)	From cystine by oxidation on chromatograms or H_2O_2 (observed at times by Payne, Fults and Hay.)	
Valine	(16)	Valine	(16)		
Isoleucine	(18)	Isoleucine	(18)		
Gamma amino butyric acid	(23)	Gamma amino butyric acid	(23)	Methionine sulphone from methionine or methionine sulphoxide by H_2O_2 and ammonium molybdate	
10 Amino acids		Threonine	(7)		
Numbers are those used by Dent *et al.* (4)		Histidine	(11)	5 unidentified spots No. 22, 24, 25, 26, 27 (No. 22 one of 2 compounds)	
		Arginine	(13)		
		Proline	(15)		
		Phenylalanine	(19)	27 spots of which only 22 were positively identified	
		Tyrosine	(21)		
		Methionine sulphoxide comes from methionine by oxidation on chromatogram during spraying or by treatment with H_2O_2	(14)	*Since the paper of Dent *et al.* (4), Steward *et al.* (23) positively identified spot 23.	
		17 amino acids			

RESULTS.—The data in table 1 show the free amino acids found in the soluble nitrogen fraction of Red McClure potato tubers. Table 1A shows the amino acids observed in two dimensional phenol-collidine chromatograms when 5 lambda portions of the filtrates, brought to original volume, were used. Table 1B lists the amino acids observed when 5 lambda portions of ⅕ volume filtrate concentrates were used. Table 1C lists the additional amino acids observed by Dent *et al.* (4). It will be noted that there are 7 more amino acids easily detected in the filtrate concentrated to ⅕ volume, than in chromatograms where the filtrate was made to volume. Of the 22 amino acids positively identified by Dent *et al.* (4) and Steward *et al.* (23), the 17 listed in table 1B were consistently found. Occasionally cysteic acid from the oxidation of cystine was seen. Tryptophan, glycine and alpha amino butyric acid were not observed in Red McClure potatoes. This may have been due to differences in variety, methods of extraction, concentration of the soluble nitrogen fraction, or to the fact that they appeared in amounts too small to chromatograph.

Table 2 shows the effect of 2,4-D treatment on the free amino acids from tubers of Red McClure potatoes. These data were obtained from one-dimensional chromatograms, using concentrated filtrates. Eight of the 9 major amino acid spots, in the treated samples, decreased significantly in relative density as measured by the Welch densichron. Spot 8, which contained glutamic acid, showed a significant increase in the treated samples, as shown in table 2. In the one dimensional chromatograms, arginine and proline were above Spot 1; histidine and tyrosine between Spots 2 and 3. They were not measured nor were methionine sulphoxide and cysteic acid. See figure 1.

DISCUSSION AND SIGNIFICANCE OF RESULTS.—In order to evaluate the significance of changes in the free amino acids, it might be well to examine some of the possible interlocking mechanisms of photosynthesis, respiration, and protein synthesis. A highly critical evaluation of this area of thought has recently been made by Steward and Thompson (22). Some of these ideas, valuable in formulating a hypothesis for the mechanism of action for 2,4-D, are:

1. Photosynthesis, respiration, and the nitrogen cycle are not isolated phenomena but are closely associated and interrelated.
2. The Krebs cycle of respiration (carboxylic acid cycle) proceeds in *green* cells both in the dark and the light. The metabolic pattern, however, is probably different under the two conditions.
3. In green cells in the light, or in storage cells in the absence of light, synthesis of protein predominates over its breakdown. The limiting factor appears to be low internal CO_2 tension. With low CO_2, the carboxylic acid cycle is maintained by carbon residues fed into it, from deaminated amino acids (other than glutamic). The sugars which, under high CO_2 tension, would go through the carboxylic acid cycle *via* pyruvate are diverted to protein synthesis. The nitrogen from the deaminated amino acids passes through glutamine or glutamic acid on its way to combine with the diverted sugars to synthesize protein.
4. Glutamic acid is regarded as the ultimate donor of nitrogen actually incorporated into protein (Steward and Street, 21). All nitrogen

TABLE 2.—*The effect of 2,4-D treatment on the free amino acids in tubers of Red McClure potatoes.**

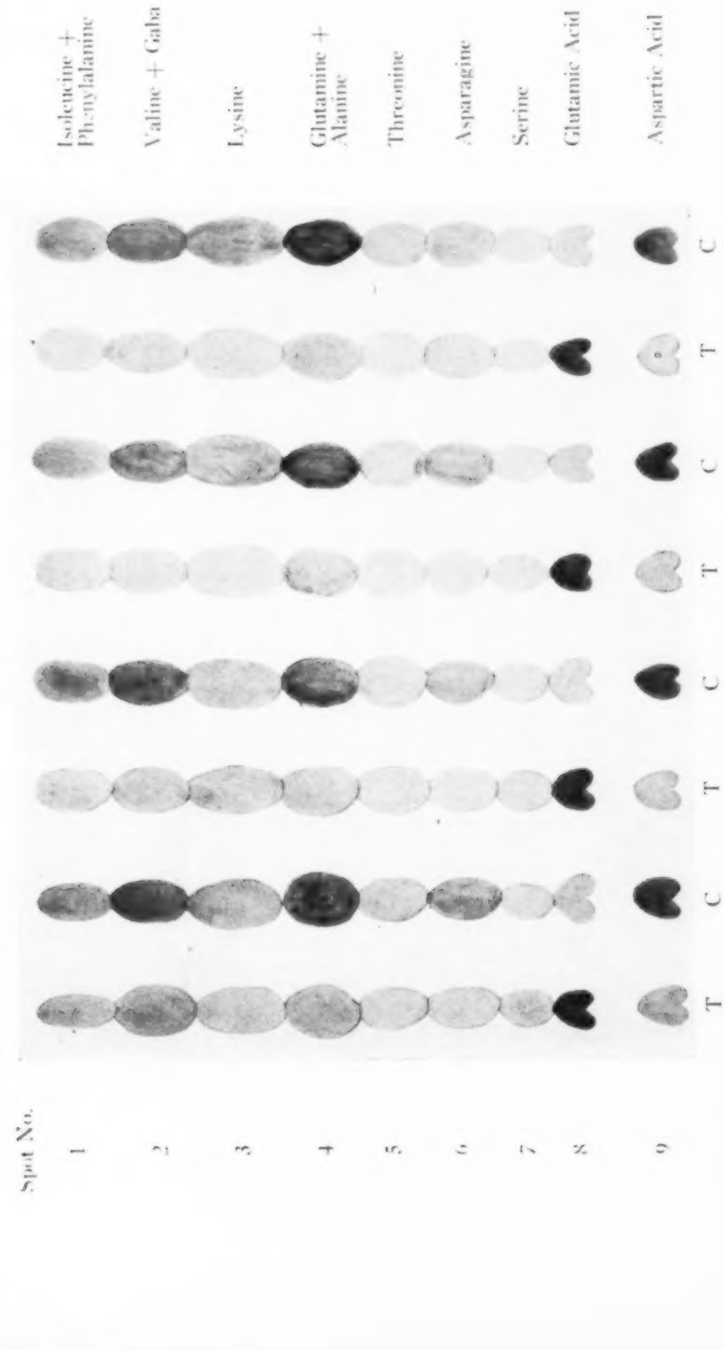
Spot No.	Amino Acids	Mean Densichron Units		Diff-erence	Stand-ard Devia-tion	Stand-ard Error	Minimum Difference Required for Significance	
		Treated	Control				.05	.01
1	Isoleucine Phenyl-alanine	1.63	1.95	—0.32	0.13	0.19	0.03	0.04
2	Valine Gamma amino butyric acid	2.06	2.35	—0.29	0.18	0.26	0.14	0.19
3	Lysine	1.46	1.58	—0.12	0.14	0.20	0.11	0.15
4	Glutamine Alanine	2.47	2.82	—0.35	0.14	0.20	0.11	0.15
5	Threonine	1.40	1.58	—0.18	0.10	0.15	0.08	0.11
6	Asparagine	1.78	1.93	—0.15	0.10	0.14	0.08	0.11
7	Serine	1.46	1.56	—0.10	0.03	0.12	0.08	0.11
8	Glutamic acid	2.68	2.47	+0.21	0.15	0.21	0.12	0.16
9	Aspartic acid	2.07	2.17	—0.10	0.12	0.13	0.09	0.12

*Arginine, proline, histidine, tyrosine, methionine sulphoxide and cysteic acid, although identified by 2-dimensional chromatograms, appeared in concentrations too small to measure.

Concentrated filtrates were used on 1-dimensional chromatograms for this table, and mean densichron values shown represent 14 determinations or each amino acid spot.

from other free amino acids has been regarded as being canalized through either glutamine or glutamic acid enroute to protein. These reactions involve either oxidative deaminations or transaminations (Steward and Street, 21).

If one accepts these ideas in connection with the known accelerating effects of 2,4-D on CO_2 production in storage tissues (Rasmussen (13), there is a basis for interpreting free glutamic acid accumulation and other free amino acid depressions in potato tubers from plants treated with 2,4-D. The probable high internal CO_2 production in the tubers could act to suppress protein synthesis in the usual manner because of the diversion of sugars *via* pyruvate into the carboxylic acid cycle. With this diversion, nitrogen would be expected to accumulate as glutamic acid (see figure 2).



T=Tuber juice samples from plants sprayed with 1/3 lb. per acre of NA 2,4-D.
C=Control samples from untreated plants Each sample was 1λ in volume.
Fig. 1.—Diagram of one-dimensional chromatograms developed in 80 per cent phenol.

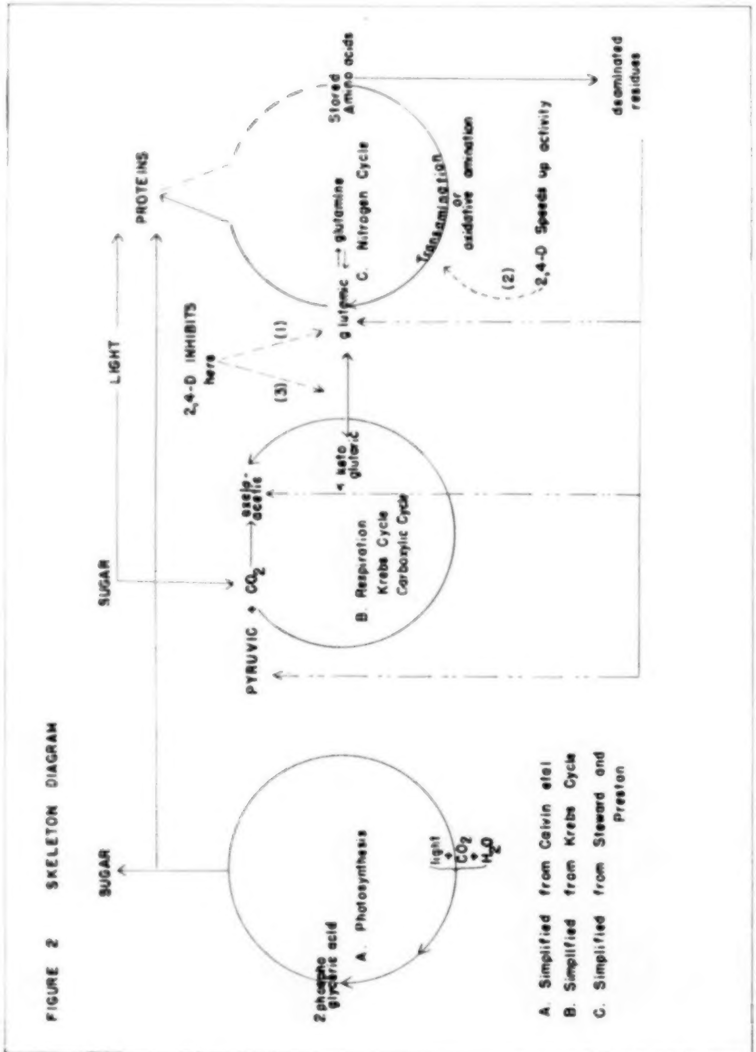


Fig. 2.—Skeleton diagram.

Since a significant decrease in all free amino acids, except glutamic acid, was found, it might be assumed that the 2,4-D increased the activity of the specific oxidative deaminases and transaminases involved. This would be expected according to Eyster's (6) theory of 2,4-D action, *i.e.*, that it acts to free enzymes from their bound substrates and make them more active.

Summary

Treatment of Red McClure potato plants with low concentrations of the sodium salt of 2,4-D results in an increase of free glutamic acid and a decrease of 11 other amino acids. Possible mechanisms of 2,4-D action have been discussed in relation to these facts.

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